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INTRODUCTION: Medical devices intended for implantation into humans require sterilisation using regulatory approved methods. Within a research lab setting however, this sterilisation process often incorporates non-approved techniques because they are considerably cheaper and have a quick turnaround. This study compared the in vivo biological response of electrospun poly(ε-caprolactone) fibre yarns fabricated by two different manufacturers and subsequently sterilised by irradiation (approved) or ethanol submersion (non-approved). The efficacy of the implant was further compared to autograft - the current gold standard treatment for tendons requiring reconstruction.

METHODS: Electrospun poly(ε-caprolactone) (PCL) yarns were manufactured at two separate sites – in-house (UOM) and The Stellenbosch Nanofiber Company (SNC). Yarns were sterilised by either gamma irradiation (25 kGy, Synergy Health) or submersed in increasing concentrations of ethanol (70-100 % w/v) and washed in phosphate buffered saline solution. Individual yarns were implanted into purpose-made defects within the flexor digitorum longus tendon of mice and held in place by single-knot sutures at either end (*Fig.1A*). Yarns were compared to autograft at 3, 21 and 42 days (n=4). Cell infiltration (migration into and along the graft), cell proliferation (cell coverage within implant area) and immunohistochemistry staining for 4 key processes was performed: (1) inflammation (CD45, F4/80, Ly6G); (2) collagen production (Hsp47); (3) cell turnover (BrdU, TUNEL); and (4) blood vessel formation (CD31, SMA). Staining was semi-quantified from serial tissue sections (5 µm thick) using a maximum +++ or --- score system to indicate the level of positive staining compared to the control.

RESULTS: Cells were observed to have infiltrated all electrospun yarns at 3 days and continued to migrate through to the centre of the implant with time, >90% at 42 days (*Fig.1B*).

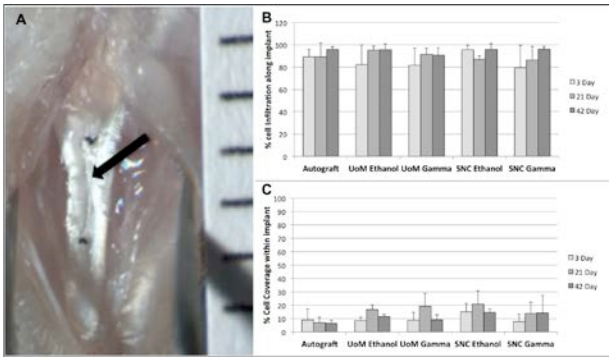

Fig. 1: (A) Arrow indicates position of electrospun yarn in situ. Black dots represent suture knots. (B) % cell infiltration along the implant and (C) % cell coverage within the implant for all electrospun yarns compared to autograft.

Cells colonised a low percentage of the total implant area for all yarns implanted, though cell coverage was comparable to the autograft tissue (*Fig.1C*). Collagen production was similar to the autograft control for all yarns over time (*Table 1*).

Table 1. Level of immunostaining for Hsp47 (collagen production) for implant area compared to autograft (0 = no observed difference).

Hsp47	3 day	21 day	42 day
UOM EtOH	0	0	0
UOM Gamma	0	+	0
SNC EtOH	0	0	0
SNC Gamma	0	0	0

DISCUSSION & CONCLUSIONS: Irrespective of manufacturer and sterilisation technique, the biological response of the electrospun yarns in vivo was similar to the current gold standard, autograft. This would suggest ethanol submersion, as a sterilisation technique is acceptable when undertaking short-term in vivo animal studies.

REFERENCES: [1] L.A. Bosworth, A. Gibb, S. Downes (2012) *J Polym Sci Part B Polym Phys* **50**:870-876.

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